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Structural Basis for Triclosan and NAD Binding to Enoyl-ACP Reductase of *Plasmodium falciparum*

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Recent discovery of type II fatty acid synthase in the malarial parasite *Plasmodium falciparum* responsible for the most debilitating form of the disease in humans makes it ideal as a target for the development of novel antimalarials. Also, the identification of the enoyl-acyl carrier protein reductase from *P. falciparum* and the demonstration of its inhibition by triclosan [5-chloro-2-(2,4-dichlorophenoxy)phenol], a potent antibacterial compound, provide strong support for the above. In the studies reported here, a model of the enzyme in complex with triclosan and the cofactor NAD has been built by homology modeling with a view to understand its binding properties and to explore the potential of triclosan as a lead compound in designing effective antimalarial drugs. The model indeed provided the structural rationale for its interaction with ligands and the cofactor and revealed unique characteristics of its binding site which could be exploited for improving the specificity of the inhibitors.

Key Words: **enoyl-ACP reductase; FabI; *Plasmodium falciparum;* molecular model; triclosan; NAD binding.**

Despite enormous efforts, malaria remains one of the most devastating of the diseases of the tropics, accounting for over 500 afflictions from which 2.5 million deaths occur annually (1). The disease also exacts enormous toll in terms of the lost manpower and medical expenses. The most severe form of the disease is caused by *Plasmodium falciparum* (2). Many different drugs have been used for treating malaria including quinine, atabrine, chloroquine, mefloquine, and primaquine (3). The drugs work in the patient by killing the parasite at various stages of its life cycle. Moreover, the currently used drugs either act by poorly understood mechanism or act on targets that overlap with the biochemical machinery of the host (3–5). They, therefore, are responsible for many harmful side effects. Also, the parasite resistance to these drugs is occurring at an alarming rate (6, 7). Evolution of resistance to the common antimalarial drugs makes drug development for treating malaria a significant scientific challenge. In view of all the above, fresh avenues for treating malarial infections are tremendous.

Of the numerous interventional strategies, one approach is to target the parasite's unique metabolic machinery. Recently, we demonstrated that *P. falciparum* contains type II fatty acid synthase (FAS), wherein each of the steps of fatty acid synthesis are catalyzed by independent enzymes (8–10). In contrast, the human host utilizes type I FAS in which each of the reaction of fatty acid synthesis are carried out by individual domains that are part of the very huge multifunctional proteins (8, 11). This fundamental difference in the organization of the *de novo* fatty acid synthesis system between the malarial parasite and its human host can be taken advantage of for the development of novel antimalarial agents (8). Indeed interruption of the operation of the *de novo* fatty acid synthesis pathway of the parasite by triclosan (Fig. 1), a hydroxydiphenyl ether class of biocide used widely in human consumer products, compromised parasite growth completely (8, 12). Both the chloroquine sensitive and resistant strains were highly susceptible to the inhibitory effect of triclosan (8). More so, the biocide was able to rescue malaria afflicted mice from certain death as well as prolong their survival for a number of weeks (8).

Enoyl-acyl carrier protein reductase (FabI) carries out the stereospecific reduction of α,β-unsaturated double bonds of the fatty acids bound to the acyl carrier protein in an NADH or NADPH dependent reaction. Like its bacterial counterparts, triclosan was shown to inhibit FabI, a key enzyme of FAS II, by directly binding to the enzyme and promoting the interaction with the oxidized co-factor, NAD, thus bringing the fatty acid synthesis and cell growth to a complete stop (8,

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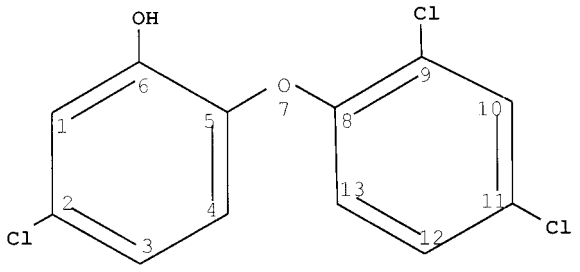
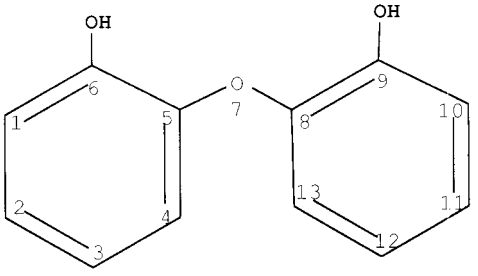


5-Chloro-2-(2,4-dichlorophenoxy)phenol (Triclosan)



2,2' Dihydroxydiphenyl ether

FIG. 1. Chemical structures of triclosan and 2,2′-dihydroxydiphenylether which are shown to inhibit the growth of *Plasmodium falciparum* in culture with IC_{50} values of 0.7 and 1 mM, respectively.

13–15). Also unlike other organisms, including plants, *Plasmodium* was shown to rely on NADH rather than NADPH as the preferred co-factor for both fatty acid synthesis as well as the reaction catalyzed by FabI (16–18).

The gene for FabI was identified from an unannotated and unfinished sequence deposited in the Plasmodium database released by the Sanger Center as well as sequenced in our laboratory (8, 19). Certain unique features of the *plasmodium* FabI sequence vis a vis its counterpart in other organisms were also reported. To aid in the rational design of drugs based on hydroxydiphenyl ether nucleus and to find the molecular basis for the binding of triclosan and the cofactor NAD, we built a model of the enoyl-ACP reductase of *Plasmodium falciparum* by homology modeling and studied the enzyme–ligand interactions. The model clearly explains the observed affinities of the enzyme toward the inhibitors and the cofactor and also reveals certain unique structural features of the ligand binding region that will aid in designing more specific antimalarial drugs.

METHODS

The coordinates of the enoyl-ACP reductase from *Brassica napus* in complex with NAD and triclosan (20) have been obtained from the protein data bank (code: 1D7O). There is 55% sequence identity and 71% homology between the two enzymes in the region where the crystal structure of the *B. napus* enzyme is available. Structure of the *Brassica* enzyme is available from the 83rd residue. The plasmodium enzyme has 86 residues corresponding to these 82 residues without any sequence homology. Only these two enzymes seem to be having this N-terminal extra region. Plasmodial enzyme also has an additional long stretch of 43 residues (starting from residue 330) which is unique to this enzyme. Except for this region and a few differences at the termini, the two sequences align very well without any insertions or deletions (an alignment of a few enoyl-ACP reductases was given in Ref. 8). The model for the *Plasmodium* enzyme was built (Fig. 2) using the program SYBYL version 6.6 on an O2 work station (Silicon Graphics, U.S.A.) using the COMPOSER module. Energy minimization was carried out using Powell minimization (21) in which gradient descent minimization is used for convergence.

RESULTS AND DISCUSSION

Conservation of the Crucial Residues

Examination of the triclosan binding region revealed that the key residues Y282, M286, and F373 (numbering as in *P. falciparum* sequence) that interact directly with triclosan in both *B. napus* and *Escherichia coli* enzymes are conserved in plasmodial enzyme as well (Fig. 3). Also conserved is the residue Lys290 implicated in the catalytic mechanism of the enzyme along with the residue Tyr282 (20, 22–24). In addition, all the atoms within 5 Å of triclosan are exactly identical in both the enzymes. However, the occurrence of a mutation of a mostly conserved methionine residue in the vicinity (~5.3 Å) of triclosan to an alanine (377) in *P. falciparum* can be effectively made use of to design specific drugs for malaria. This is a potential site which can be exploited for introducing larger substitutions and/or incorporating additional groups at the C2 position of triclosan in attempting to design specific inhibitors targeted to the malarial enzyme. The residue (218) corresponding to Ala138 of the *B. napus* enzyme is conserved in *P. falciparum* (Fig. 3) but is replaced by a glycine, located on a loop which is 2 residues shorter than the other two in the *E. coli* enzyme. This residue interacts directly with triclosan and a mutation of glycine to valine in the *E. coli* enzyme was shown to weaken the binding of triclosan (23). The model of the *Plasmodium* enzyme suggests that this residue is critical for triclosan binding to the malarial enzyme, since replacing Ala with Gly or Val will reduce the affinity for triclosan binding because of the loss of interactions with Cβ in the case of Ala→Gly substitution

FIG. 2. Model of the *P. falciparum* enoyl-ACP reductase shown with triclosan (black) and NAD (light gray). Location of the long insertion is shown as a dashed line.

and due to steric clashes in the case of Ala→Val substitution.

The major difference in the crystal structures of the complexes of the enzyme with triclosan and NAD in *B. napus* and *E. coli* is the conformational change (20) of a loop preceding the conserved residue F373. This is the exact location of the insertion of 43 residues in the plasmodial enzyme. Since the loop is already known to be flexible, the longer loop of the *P. falciparum* enzyme can be accommodated at this position without causing any change in the rest of the structure.

Reasons for the Preferred Affinities of the Ligands

We have also examined the possible binding of NAD and NADP to the plasmodial enzyme. The only difference in the residues that interact with NAD has been found to be the change from Gly in *B. napus* to Val134 in *Plasmodium* (Fig. 4). This prevents/reduces the binding of NADP to the plasmodial enzyme because of the steric clash between the side chain of the valine and the phosphate group of NADP. This explains the higher affinity of the enzyme to NAD than to NADP (8). Chlorine substitution at C9 position of triclosan makes favorable interactions with NAD and the protein (14 interactions with NAD and 5 with the protein within 5 Å) and replacing it with an OH group will make the binding weaker. Loss of these favorable contacts is one of the reasons why 2,2′-dihydroxydiphenyl ether (Fig. 1) does not bind as strongly as triclosan to the plasmodial enzyme compared to the FabI from *E. coli*. The other reason for the very poor interaction of 2,2′-hydroxydiphenyl ether to the plasmodial FabI is the loss of chlorine substitution at C11 which has 17 favorable interactions with the protein atoms.

The affinity for triclosan in the case of the *E. coli* enzyme is twice that for 2,2′-dihydroxydiphenyl ether (14) whereas it increases 1000-fold in the case of *P. falciparum* enzyme (8). This could arise as a consequence of slightly different conformations of triclosan in the two complexes. The 2,4-dichlorophenoxy ring of triclosan turns by 16° in the *B. napus* complex compared to that in *E. coli* (23, 24) the reason being the difference in the structures at Ala218 (Ala138 in *B. napus* and Gly93 in *E. coli*). This conformational difference leads to differences in the interactions involving the chlorine atom substi-

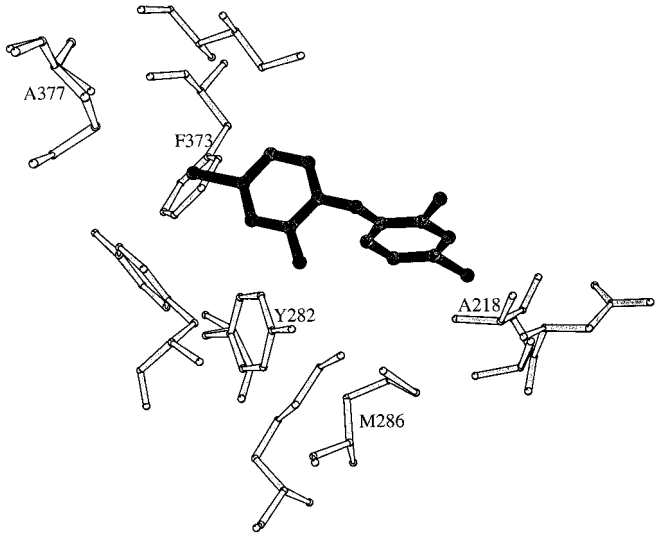


FIG. 3. Triclosan binding region in the model of *P. falciparum* enoyl-ACP reductase. Met250 of the *B. napus* enzyme is replaced by an alanine (A377) in the plasmodial enzyme.

tuted at C9 in triclosan in the crystal structures of the two complexes. Most of the van der Waals interactions less than 3.6 Å between the chlorine atom and the protein (Ala196 O, Gly93 O) and between chlorine and NAD (NO2, NC2, and NC3 atoms of the nicotinamide ribose; and the phosphate oxygen atom, NO5, attached to the nicotinamide ribose) are retained even if this chlorine is replaced by an OH group in the *E. coli* enzyme. In the *Plasmodium* enzyme complex built based on the crystal structure of *B. napus,* the contacts between chlorine are different (to AC5 of adenosine ribose; to the phosphate oxygen atom, NO5, attached to the nicotinamide ribose; and to the oxygen atom, O3, between the phosphate groups) which are lost when an OH group takes the position of CI16 thus resulting in a much higher preference for triclosan in this case compared to the *E. coli* enzyme.

CONCLUSIONS

By way of building a model of the enoyl-ACP reductase of *P. falciparum,* we have found reasons for the binding of triclosan to the enzyme and also the preferred affinity of the enzyme toward NAD compared to NADP. The present analysis has provided not only explanations for the observed binding properties of the enzyme at the atomic level but also a platform for developing antimalarials by a rational approach.

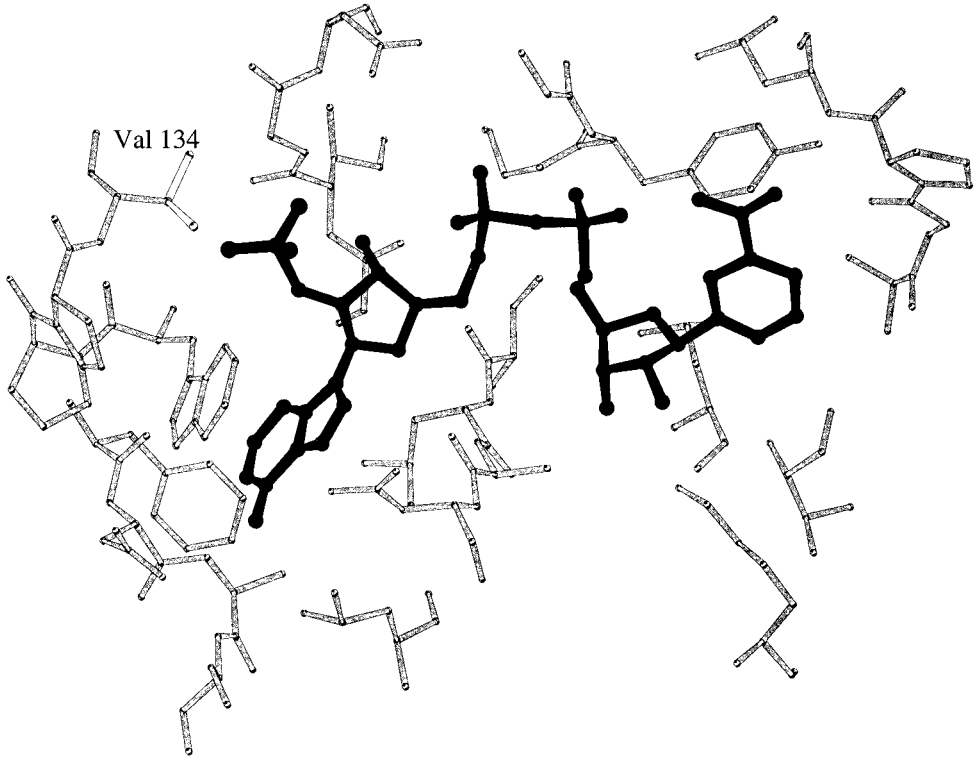


FIG. 4. Residues of the NAD (black) binding site of *P. falciparum* enoyl-ACP reductase model. Steric interference of Val134 with the modeled phosphate group prevents the binding of NADP to the enzyme.

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